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CITATION:

Nishizawa, Yoshio. URINARY γ -GTP ACTIVITY AS A DIAGNOSTIC AID FOR DIFFERENTIAL DIAGNOSIS OF RENAL TUMORS. 泌尿器科紀要 1982, 28(9): 1099-1102

ISSUE DATE:

1982-09

URL:

<http://hdl.handle.net/2433/123177>

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URINARY γ -GTP ACTIVITY AS A DIAGNOSTIC AID FOR DIFFERENTIAL DIAGNOSIS OF RENAL TUMORS

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The excreted γ -glutamyltranspeptidase (γ -GTP: EC. 2.3.2.2.) of patients with renal tumor was measured. The γ -GTP activity in the tissue and urine obtained from patients with renal cell carcinoma was significantly lower than normal. On the other hand, patients with benign renal tumors (low grade of papillary carcinoma or adenoma) had significantly higher γ -GTP activity. These results suggest that measurement of the enzyme activity of γ -GTP in urine can be used for the differential diagnosis of renal tumors.

Key words: γ -GTP, Renal tumor, Malignant renal tumor, Papilloma, Adenoma

INTRODUCTION

To the present, urinary enzymology has had little diagnostic value, being satisfactory only as a screening test because: (1) there are no specific enzymes for definite renal disorders; (2) most studies deal with only a limited number of enzymes; and, (3) tissue patterns from pathologic human kidneys are unknown. Recently, we devised a method of laboratory examination for measurement of the activity of urinary γ -glutamyltranspeptidase (γ -GTP, EC: 2.3.2.2.)^{1,2)}. We have shown that there is an important relationship between urinary γ -GTP activity, creatinine clearance and PSP (15 min) values, and that urinary γ -GTP activity can be used as a diagnostic aid for various renal diseases and renal toxicity of administered aminoglycoside antibiotics³⁻⁷⁾. We have measured the urinary γ -GTP activity of patients with malignant or benign renal tumors to examine the possibility of using urinary γ -GTP activity in diagnosing renal tumors.

MATERIAL AND METHOD

Material

A 24-hour urine sample was collected

from 30 healthy subjects (12-70 years old, male : female=15 : 15), 10 patients with renal cell carcinoma (26-67 years old, male:female=7:3), 9 patients with low grade of papillary carcinoma at renal pelvis (15-60 years old, male:female=5:4) and 6 patients with renal adenoma (13-52 years old, male:female=3:3) without the use of antiseptics. The urinary samples were well mixed before assay of γ -GTP.

Cortical parts of the kidney were obtained from 5 healthy subjects free of any renal disease who had died in traffic accidents (34-70 years old, male:female=3:2) and the same patients who had renal tumors as described above.

Homogenization of kidney

Kidney tissue was obtained immediately after nephrectomy. After routine pathological examinations, about 200-300 mg of kidney tissue was removed, and the connective tissue and hemorrhagic lesions were taken from the outer zone of the medulla. Tissues were homogenized three times for 15 sec. in a cold 0.05 M tris hydromethyl aminomethane buffer at pH 7.4 and 4°C by a homogenizator at 2,000 r.p.m. for 15 sec. The homogenate was centrifuged at 18,000g for 30 min at 4°C. Then, the supernatant was obtained.

Reagents

The tris-base was purchased from Sigma Chemical Co. (St. Louis, Mo.). The γ -GTP enzyme activity assay kit was obtained from Sankyo-Miles Co. (Tokyo, Japan).

Biochemical analysis

The γ -GTP activity was determined using a γ -GTP enzyme assay kit in the same way as described previously¹⁻³⁾.

Solution to stop the enzyme reaction was 364.61 mg of HCl dissolved in 10 ml of distilled water; the substrate solution was 1.0 ml of γ -glutamyl-p-nitroanilide; and the standard solution was p-nitroaniline.

One ml of the substrate solution was placed in the test tube and preincubated for 5 min at 37°C. After addition of 50 μ l of the urinary sample to the test tube and thorough mixing, the tube was incubated for 30 min at 37°C. Then 5 ml of the stopping solution was added, and mixed well. The mixture was immediately cooled to room temperature. As the control, a mixture of 1.0 ml of substrate, 5 ml of the stopping solution and 50 μ l of the urinary sample was used. The absorbance of the test sample(A) and control(B) was measured with a spectrophotometer (Hitachi Model 104 Tokyo, Japan) at 410 μ m in cubettes against a water blank. The γ -GTP activity was determined by measurement of absorbance at 410 μ m in a photospectrometer using

p-nitroaniline as a standard. One unit of γ -GTP was defined as the activity that catalyzed the liberation of 1 μ mole p-nitroaniline per min under the given assay condition. The urinary activity with the unit per ml of urine was expressed as mU/ml. For the standardization of the activity, 1 ml of the standard solution was placed in a test tube. The absorbance of the mixture(S) was measured consequently, the unit of the enzyme activity of the urinary sample was calculated as follows:

$$\text{urinary } \gamma\text{-GTP activity (mU/ml)} = \frac{A-B}{S} \times 250$$

The urinary γ -GTP activity was expressed by U/day. The urinary γ -GTP activity in each subject was expressed by the mean value \pm one standard error of measurements for 5 consecutive days.

The γ -GTP activity in the supernatant of the renal homogenates was measured by the same method as that described in the measurement of urinary γ -GTP activity. Renal γ -GTP activity was shown in mU/g wet tissue.

Results

The γ -GTP activity in the renal tissues and urine of the patients with renal cell carcinoma dropped significantly. Urinary γ -GTP activity in 30 healthy control

Table 1. Urinary γ -GTP activity obtained from healthy subjects and patients with various renal tumor

	Case	Age	Urinary γ -GTP activity	t-value
	(No.)	(years old)	(u/day)	
Healthy subjects	30	12-70	25.3 \pm 3.0 ²⁾	
Patients with malignant renal tumor	10	26-67	11.3 \pm 4.3	t \leq 0.05
Low grade of papillary carcinoma	9	15-60	81.7 \pm 9.0	t \leq 0.0005
Patients with benign renal tumor				
Adenoma	6	13-52	76.2 \pm 6.1	t \leq 0.0005

1) The assay method of γ -GTP activity in urine was described previously.

2) The value was showed m \pm S.D.

Table 2. γ -GTP activity in renal tissue from healthy subjects without renal disease and patients with various renal tumors

	Case (No.)	Age (years old)	Kidney tissue γ -GTP activity (mU/gr. wet tissue)	t-value
Healthy subjects	5	34-70	27.7 \pm 9.2 ¹⁾	
Patients with malignant renal tumor	10	26-67	4.1 \pm 1.9	t \leq 0.0005
Low grade of papillary carcinoma	9	15-60	81.7 \pm 9.0	t \leq 0.0005
Patients with benign renal tumor				
Adenoma	6	13-52	76.2 \pm 6.1	t \leq 0.0005

1) The detailed assay method was described in the text

2) The value was showed m \pm S.D.

	Urine	Renal tissue
Malignant tumor	↓↓	↓↓
Low grade of papillary carcinoma	↑↑	↑↑
Benign tumor	↑↑	↑↑
Adenoma	↑↑	↑↑

Table 3. γ -GTP activity in patient with various renal tumors

subjects was 25.5 ± 3.0 U/day (mean \pm SD), whereas that in 10 patients with renal cell carcinoma was 11.3 ± 4.3 U/day. The γ -GTP activity in the renal tissue obtained from 5 patients without renal diseases was 27.7 ± 9.2 mU/g of wet tissue, but that in the renal tissue obtained from 10 patients with renal cell carcinoma was 4.1 ± 1.9 mU/g of wet tissue (Tables 1 and 2). In contrast to these malignant cases, all the patients with benign renal tumors had significantly high urinary γ -GTP activity low grade of papillary carcinoma 81.7 ± 9.0 U/day; adenoma: 76.2 ± 6.1 U/day (Table 1). In these renal tumor tissue cases, the γ -GTP activity in the renal tissue was significantly elevated (papilloma: 35.6 ± 7.4 mU/g of wet tissue; adenoma: 33.9 ± 6.1 mU/g of wet tissue) (Table 2). The results obtained from these experiments are summarized in the schema (Table 3).

DISCUSSION

Recently, we devised a method for

measuring the activity of urinary γ -GTP^{1,2)}. In our previous studies on urinary γ -GTP activity we found that there is an important relationship between urinary γ -GTP, creatinine clearance and PSP (15 min) values, and that urinary γ -GTP could be used as a diagnostic aid for various renal diseases and for the renal toxicity of administered aminoglycoside antibiotics³⁻⁷⁾.

In this study, we compared the urinary γ -GTP activity in patients with various renal tumors. The γ -GTP activities in the tissue and urine of patients with renal cell carcinoma were significantly lower than normal. On the other hand, there was a highly significant increase in the γ -GTP activity of patients with benign renal tumors papillary carcinoma (papilloma or adenoma). These results suggest that renal tumors can be differentially diagnosed from the γ -GTP activity in the urine.

γ -GTP is mainly concentrated in the brush border of rat⁹⁾ and human kidneys¹⁰⁾. Moreover, our biochemical and immunological analyses have shown that the urinary γ -GTP derived from the kidney is different from that in the serum^{11,14,5,11,12)}.

Therefore, measurements of urinary γ -GTP activity might accurately express renal function. Clinically, similarities in the property of the renal tissue and urinary γ -GTP activity in patients with malignant or benign renal tumors is striking in that

differential diagnosis of renal tumors is enabled.

It is yet unknown whether the drop in γ -GTP activity in renal tumor tissues and urine of patients with renal cell carcinomas is caused by a decrease in activity or by an inhibitor in the urine^{13,14}, or whether one form of the enzyme is converted into another in certain circumstances¹⁵. The nature of the tumor may cause the drop in γ -GTP activity in malignant renal tissue and its increase in benign renal tissue (papillary carcinoma and adenoma). Although the mechanism causing this difference is still unknown, it is an important clinical finding that will be helpful in the differential diagnosis of renal tumors.

ACKNOWLEDGEMENT

Thanks are due to Miss Kanae Takahashi and Miss Yuko Fujisawa for their secretarial assistance and help.

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(Accepted for publication, March 29, 1982)

和文抄録

尿中 γ -glutamyltranspeptidase 活性測定による腎臓腫瘍鑑別診断

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西 澤 芳 男

腎臓腫瘍の悪性, 良性鑑別診断の一助とする目的で尿中 γ -glutamyltranspeptidase (γ -GTP) 活性を測定した。正常健人では尿中 γ -GTP 活性は 25.3 ± 3.0 U/day であったのに対し, 腎悪性腫瘍では 11.3 ± 4.3 U/day, 良性腫瘍である papillary carcinoma of low grade では 81.7 ± 9.0 U/day, adenoma では 76.2 ± 6.1 U/day と, 悪性腫瘍では低値, 良性腫瘍では高値をしめし, 手術よりえた腎組織中 γ -GTP 活性においても, 腎疾患を有さないコントロールでは 27.7 ± 9.2 mU/gr,

wet tissue であるのに対し, 悪性腫瘍例では 4.1 ± 1.9 mg/gr, wet tissue, papillary carcinoma of grade Papilloma では 81.6 ± 9.0 mg/wet tissue, adenoma では 66.2 ± 6.1 mU/gr, wet tissue と腎細胞腎の組織では低値, 良性腫瘍組織では高値をしめし, 尿中 γ -GTP 活性と良好な相関をしめした。以上の結果より腎腫瘍の悪性, 良性鑑別診断に尿中 γ -GTP 活性測定が有用である可能性が示唆された。